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SCREENING OF BIOACTIVE COMPOUNDS OF GREEN TEA (*CAMELLIA SINENSIS*)

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ABSTRACT

Green tea is one of the most ancient and popular therapeutic beverages consumed around the world. This product is made from the leaf of the plant called '*Camellia sinensis*'. It can be prepared as a drink, which can have many systemic health effects or an 'extract can be made from the leaves to use as medicine. Medicinal plants are important sources of phytochemicals which are crucial in the treatment of various diseases. *Camellia sinensis* have been used in globally as medicinal plants to treat various ailments. In this study, the leaves of *Camellia sinensis* were subjected to phytochemical screening and the bioactive components was also determined by GC-MS analysis. Twenty compounds were identified which are almost contributed by polyphenols which plays key role in prevention and treatment of many diseases.

KEYWORDS: *Camellia Sinensis*- Green tea, Phytochemical, Bioactive components.

INTRODUCTION

World Health Organization estimate over 80% of the people in developing countries depend on traditional medicines for their primary health needs.[1] India is one of the largest producers of medicinal herbs and is rightly called the botanical garden of the world as it is sitting on a gold mine of well-recorded and traditionally well practiced knowledge of herbal medicine. About 17,000 species of Indian flora about 7500 species of higher plants are reported to possess medicinal value and in other countries it is projected about 7% and 13%. There are estimated to be around 25,000 effective plant-based formulations, used in folk medicine and known to rural communities in India.[2] The search for new molecules, nowadays, has taken a slightly different route where the science of ethno botany and ethno pharmacognosy are being used as guide to lead the chemistry towards different sources and classes of compounds.[3] Plant derived natural products hold great promise for discovery and development of new pharmaceuticals.[4] WHO has considered phytotherapy in its health programmed; because these drugs are safe, cost effective and most importantly people have faith in them. The demand for crude drugs has undergone a considerable change in recent years due to aggressive marketing of the crude drugs. The novel molecules from plant sources have been instrumental in development of structurally modified compounds, which assist a lot in the development of modern therapeutic system.[5] Phytochemicals are responsible for medicinal activity of plants and these biochemicals are naturally accruing in the plants that have defense mechanisms and protect from various diseases.[6] The phytochemical are very important in medicine and constitute most of the valuable drugs.[7] This biochemicals are often referred to as secondary metabolites which is useful to traditional medicine system are identified by GC-MS technique.[8] In recent years Gas Chromatography –Mass Spectrum (GC-MS) studies have been increasingly applied for the analysis of medicinal plants as this technique has proved to be a valuable method for the analysis of essential oil, alcohols, acids, esters, alkaloids, flavonoids, steroids, amino and nitro compounds.[9] The green tea is obtained from the tea plant *Camellia Sinensis* belongs to the family Theaceae. Green tea is one of the most consumed drink in the world after water. It is generally safe, nontoxic, and has no side effects after consumption.[10] The aim of the present study in qualitatively screen and identify the major phytochemicals groups of Green tea, *Camellia Sinensis.*

MATERIAL AND METHODS

Sample collection

The fresh leaves of *Camellia sinensis* (leaves) were collected from the dense tea state garden, Ooty, Tamilnadu. South india. The leaves were washed in clean water and air dried in room temperature. The dried plant materials were milled to a fine powder using grinder and stored in the dark at room temperature in airtight containers.

Sample Authentication

Camellia Sinensis was identified and authenticated by Botanist, Dr. Soosai Raj, M.Sc., Ph.D., Department of

Botany, St. Joseph's College, Tiruchirappalli, Tamil Nadu, India.

Botanical Description

Camellia sinensis is native to mainland china, South and Southeast Asia, but it is today cultivated across the world in tropical and subtropical regions. It is an evergreen shrub or small tree that is usually trimmed to below two meters (six feet) when cultivated for its leaves. It has a strong taproot. The flowers are yellow –white, 2.5-4 cm in diameter, with 7 to 8 petals. The leaves are 4-15 cm long and 2-5 cm broad. The young, light green leaves are preferably harvested for tea production; they have short white hairs on the under side. Older leaves are deeper green. Different leaf ages produce differing tea qualities, since their chemical compositions are different. Usually the tip (bud) and the first two to three leaves are harvested for processing. This hand picking is repeated every one to two weeks.

Preparation of plant extracts

50 g of dried powder of plant was extracted with ethanol by continuous hot percolation, using soxhlet apparatus and concentrated up to 50 ml in desiccators under reduced pressure. The concentrated extract were lyophilized and used for further studies.

Qualitative Phytochemical Analysis

Chemical tests were carried out on the alcoholic extract and on the powdered specimens using standard procedures to identify the constituents as described by Sofowara, Trease and Evans and Harborne.

Quantitative analysis of Phytochemicals

Determination of total phenols by Spectrophotometric method. Flavonoid determine by the method of Bohm and Kocipai-Abyazan. Saponin determine by the method of Obadoni and Ochuko.

GC-MS Analysis

GC-MS analysis was carried out on Shimadzu 2010 plus comprising a AOC-20 I auto samples and gas chromatograph interfaced to a mass spectrometer instrument employing the following conditions; column RTX 5Ms (Column diameter is 0.32 mm, column length is 30m, column thickness 0.50 µm), operating in electron impact mode at 70 e V; Helium gas (99.999%) was used as carrier gas at a constant flow of 1.73 ml / min and an injection volume of 0.5µl was employed (Split ratio of 10:1) injector temperature 270°C ; ion-source temperature 200°C. The oven temperature was programmed from 40°C (isothermal for 2 min), with an increase of 8°C /min, to 150°C, then 8°C/min to 250°C, ending with a 20 min isothermal at 280°C. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds and fragments from 40 to 450 Da. Total GC running time is 51.25 min. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas. Software adopted to handle mass spectra and chromatograms was a Turbo Mass ver 5.20.

Identification of Components

Interpretation on GC-MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained (Dr. Dukes).

RESULT AND DISSCUSSION

This present study revealed the presence of medicinally active constituents and are summarized in Table 1. The phytochemical screening of water extract of *Camellia Sinensis* showed that the presence of Alkaloids, flavonoids, phenols, tannins, saponins, steroids and terpenoids, and carbohydrate, were as Cardiac glycosides and phlobatannins and Protein in absent in water extract. Similarly Ethanol extract of *Camellia sinensis* leaves showed that the presence of Alkaloids, flavonoids, phenols, Tannins, Carbohydrate and protein. Were as Saponins, Steroids, Terpenoids, and glycosides is absent in Ethanol extract. Acetone extract also showed Alkaloids, flavonoids, Phenols, Tannins, and Saponins were as steroids, terpenoids, glycosides and phlobatannis and Carbohydrates is absent in acetone extract of *Camellia Sinensis.* Significant amount of flavonoids (168mg/gm), Phenol (300mg/gm) Alkaloids (50mg/gm), and tannins (33mg/gm). From the above results, it can be noted that successful extraction of biologically active compounds from plants like *Camellia sinensis* is largely dependent on the type of solvent used during extraction. Different solvents with differing polarities extract specific phytochemicals in plant.[11] In this study, polar solvents like water, ethanol and acetone yielded highest amount of crude extracts and also had the highest presence of phytochemicals. This study therefore validates the hypothesis that variations in solvents used will affect the presence of bioactive compounds of an extract. It also implies that the choice of a solvent is affected by different factors like class of phytochemicals, diversity and polarity of the compounds to be extracted.[12] The extracts of Ethanol and Acetone indicated the presence of alkaloids, flavonoids, phenols, and tannins. Ethanol and Acetone Occasionally tannins and terpenoids will be found in aqueous phase, but they are more often obtained by treatment with less polar solvents.[11] Qualitative screening of *Camellia Sinensis* leaves extracts have alkaloids, flavonoids, phenols, tannins, saponins, steroids, and terpenoids, carbohydrates, protein.[13]

Table-1: Phytochemical Analysis of *Camellia sinensis* leaves from different solvents extracts.

S. No	Phytochemicals	Water	Ethanol	Acetone	Quantitative analysis (mg/gm)
1	Glycosides	_	-	-	---
2	Flavonoids	+	+	+	168
3	Steroids and Terpenoids	+	-	-	---
4	Tannins	+	+	+	33
5	Saponins	+	-	+	---
6	Phenols	+	+	+	300
7	Alkaloids	+	+	+	50
8	Carbohydrates	+	+	-	---
9	Protein	-	+	+	---
10	Phlobatannins	-	-	-	---

(+) indicates presence of phytochemicals; (-) indicates absence of phytochemicals

In GC-MS analysis, totally 20 compounds identified from the methanol fractions of the *Camellia Sinensis* is presented in Table 2 and shown in fig 1. The plant samples revealed the synthesis of 1,2,5,6-Tetrahydropyridin-2-one, 5-methyl, 2,3-Pentanedione, 4-methyl -3H- Pyrazol-3-one, 2,4- dihydro -2, 4, 5-trimethyl, 1,2,5,6-Tetrahydropyridin-2-one, 5-methyl, 3-Amino-2-oxazolidinone, 4H- Pyran -4-one, 2, 3-dihydro-2-(1-(benzyloxy)ethyl), 2-Methoxyresorcinol, Eugenol, Napthalene, 6-ethyl-1,2,3,4-tetrahydro-1, 1,4,4-tetramethyl -7-(1-methylethenly), 1,2,3-Benzenetriol, Sucrose, D-Allose, Oxalic acid, 2-ethylhexyl hexyl ester, Bicyclo(3.1.1) heptan-3-ol, 2,6,6-trimethyl, 3,7,11,15-Tetramethyl-2-hexadecen-1-ol, 1HPurine-2, 6-iodine, 3,7-dihydro-1, 3, 7-trimethyl, 1H-Purine-2, 6-dione, 3, 7-dihydro-1,3-dimethyl, phytol, Myristoyl chloride and Squalene. All these compounds are of pharmacological importance as they posses the properties such as antibacterial, anti-diabetic and analgesic. Investigation of alkaloids have revealed many pharmacological properties including anti diabetic, antiprotozoal and cytotoxic[14] and anti-inflammatory properties.[15] Flavonoids and phenolic compounds in various plants have been reported to have multiple biological effects like antioxidant, free radical scavenging abilities, anti-inflammatory and anti-Carcinogenic properties.[18] Steroids in plants are known for their insecticidal, analgesic properties.[16] Tannin isolated from medicinal plants have also been reported to exhibit remarkable toxicity against bacteria and fungus.[17]

Table 2: GC-MS Analysis of *Camellia Sinensis.*

S. No	Peak name	Retention time	Peak Area	% Peak Area
1.	Name:1,2,5,6Tetrahydropyridin-2-one, 5-methyl- Formula: C_6H_9NO MW: 111	6.26	1507229	0. 4019
2.	Name: 2,3 Pentanedione,4 methyl- Formula: $C_6H_{10}O_2$ MW: 114	6.74	471922	0.1258
3.	Name: 3H-Pyrazol-3-one, 2,4-dihydro-2,4,5-trimethyl – Formula: $C_6H_{10}N_2O$ MW: 126	7.39	1086150	0.2896
4.	Name: 1,2,5,6-Tetrahydropyridin -2-one, 5-methyl- Formula: C_6H_9NO MW: 111	7.90	1455643	0.3882
5.	Name: 3-Amino-2 oxazolidinone Formula: $C_3H_6N_2O2$ MW: 102	8.50	193891	0.0517
6.	Name: 4H-Pyran-4-one,2,3-dihydro-2-[1-(benzyloxy)ethyl]- Formula: $C_{14}H_{16}O_3$ MW:232	8.60	1165119	0.3107
7.	Name: 2-Methoxyresorcinol Formula: $C_7H_8O_3$ MW: 140	10.72	844408	0.2252
8.	Name: Eugenol Formula: $C_{10}H_{12}O_2$ MW:164	12.17	486539	0.1297

9.	Name: Naphthalene, 6-ethyl-1,2,3,4-tetrahydro-1,1,4,4-tetramethyl-7-(1methylethenyl)- Formula: $C_{19}H_{28}$ MW: 256	12.91	705406	0.1881
10.	Name:1,2,3-Benzenetriol Formula: $C_6H_6O_3$ MW:126	13.36	5607421	1.4953
11.	Name: Sucrose Formula: $C_{12}H_{22}O_{11}$ MW: 342	13.99	5238841	1.3970
12.	Name: D-Allose Formula: $C_6H_{12}O_6$ MW:180	15.30	548481	0.1463
13.	Name: Oxalic acid, 2-ethylhexyl hexyl ester Formula: $C_{16}H_{30}O_4$ MW:286	17.70	11093346	2.9581
14.	Name: Bicyclo [3.1.1]heptan-3-ol,2,6,6-trimethyl- Formula: $C_{10}H_{18}O$ MW: 154	17.91	3115463	0.8308
15.	Name: 3,7,11,15-Tetramethyl-2-hexadecen-1-ol Formula: $C_{20}H_{40}O$ MW: 296	20.08	2854328	0.7611
16.	Name: 1H-Purine-2,6-dione,3,7-dihydro-1,3,7-trimethyl- Formula: $C_8H_{10}N_4O_2$ MW: 194	20.79	324399328	86.5030
17.	Name: 1H-Purine-2,6-dione,3,7-diydro-1,3-dimethyl- Formula: $C_7H_8N_40_2$ MW:180	21.18	7978280	2.1275
18.	Name: Phytol Formula: $C_{2O}H_{40}O$ MW: 296	23.23	2225372	0.5934
19.	Name: Myristoyl Chloride Formula: $C_{14}H_{27}C_{10}$ MW: 246	25.01	681042	0.1816
20.	Name: Squalene Formula: $C_{30}H_{50}$ MW:410	30.24	3356811	0.8951

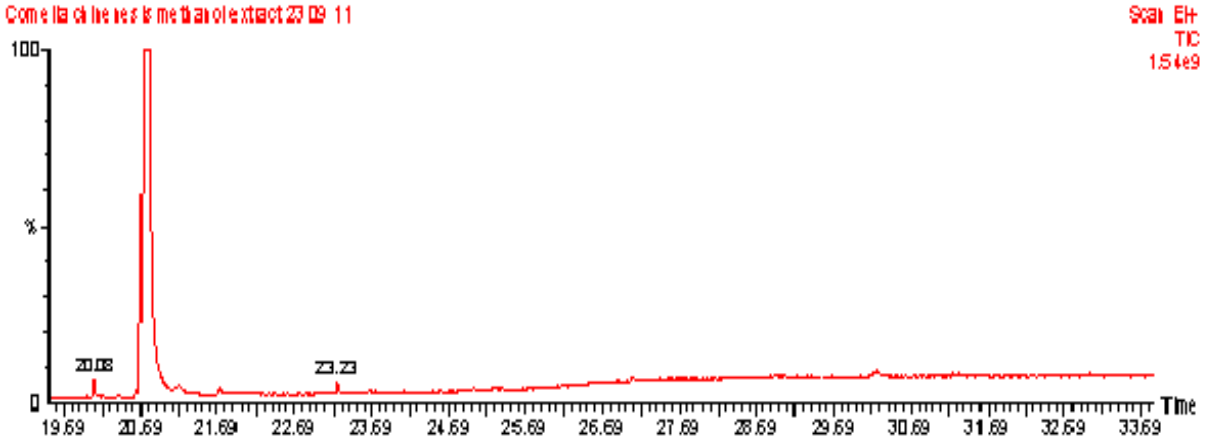

Fig 1: GC-MS analysis of *Camellia sinensis.*

CONCLUSION

Phytochemicals present in the leaves of *Camellia sinensis* indicates their potential as source of bioactive compounds that may supply novel medicines. In the present study 20 compounds from the leaves extract of *Camellia sinensis* were identified by Gas Chromatography Mass Spectrometry (GC-MS) analysis. The research findings have shown that the leaves extract of *Camellia sinensis* is extensively rich in secondary

metabolites. The plant leaves has a high potential for a vast number of bioactive compounds which justified its use for various ailments by traditional practitioners.

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